

FACTORS INFLUENCING THE ACTIVITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* TREATMENTSN. BECKER,¹ M. ZGOMBA,² M. LUDWIG,³ D. PETRIC² AND F. RETTICH⁴

ABSTRACT. Environmental factors influence the effectiveness of microbial control agents in mosquito control programs. Four of these factors (water temperature, larval density, sunlight and the effect of associated filter feeders) were studied with *Bacillus thuringiensis* var. *israelensis* under laboratory and semifield conditions in Europe using different instars of *Aedes vexans*, *Ae. aegypti* and *Culex pipiens*. Bioassays conducted at a low temperature (5°C) yielded 10-fold higher LC₅₀ and LC₉₀ values compared with those conducted at a high temperature (25°C). The efficacy of *B.t.i.* decreased in a linear manner with increasing larval density. Sunlight can reduce the effectiveness of *B.t.i.* by several times. Competition in food intake by filter feeding *Daphnia* resulted in lower mortality of mosquito larvae after *B.t.i.* applications.

INTRODUCTION

In many mosquito control programs *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) has proved to be highly efficient against many mosquito species and to be safe environmentally (Garcia et al. 1981, Schnetter et al. 1981, Mulla et al. 1982a, 1982b; Becker and Ludwig 1983, Lacey 1985, Gharib and Hilsenhoff 1988). This microbial agent has been widely used in Germany against floodwater and snow-melt mosquitoes over a period of 10 years, and has successfully controlled mosquitoes in an area of approximately 500 km² within the Upper Rhine Valley through the application of several tons of various formulations each year.

A number of environmental and biological factors play an important role in the efficacy of *B.t.i.* Experience with the routine treatment has shown that an appropriate dosage for each specific situation has both ecological and economic advantages. It is therefore of vital importance to understand the impact of the various environmental and biological factors on its efficacy.

Apart from the different susceptibilities of various mosquito species to *B.t.i.* (Aly 1983, Aly et al. 1987, Rettich 1983), there are other factors such as temperature (Wraight et al. 1981, 1987; Mulla et al. 1982a, 1990; Ignoffo et al. 1983, Lacey and Oldacre 1983), larval density (Davidson et al. 1981, Mulla et al. 1990), sunlight (Burke et al. 1983, Morris 1983) and presence of associated filter feeding non-target organisms (Rettich 1983) which could potentially influence the efficacy of *B.t.i.*

We attempted to verify in bioassays the effect of these factors in the specific conditions of Central Europe, where widespread *B.t.i.* treatments are conducted.

MATERIALS AND METHODS

All bioassays were done according to World Health Organization guidelines (WHO 1981), with slight modifications to meet the specific needs of this study: 50 mg of standard *B.t.i.* (Bactimos WP, 6,000 AAU/mg) were added to 10 ml of double-distilled water and homogenized in a mixing machine (IKA Comibag Reo) at 700 rpm for 10 min, then homogenized in an ultrasonic bath (Branson Instruments) for a further 15 minutes. One ml was taken from the homogenized solution and added to 99 ml of distilled water. Depending on the concentration required, a range of 15–1,500 µl of homogenized and diluted Bactimos WP suspension was added to the test vessels with a micropipette. Tests were run at 5 or 6 different concentrations with controls, in at least 3 replicates.

The *Daphnia* tests were carried out with a *B.t.i.* flowable concentrate (Teknar HP-D, 1,500 AAU/mg) and a powder formulation (Teknar TC, 15,000 AAU/mg). The mortality rate was evaluated after 24 and 48 h and corrected according to Abbott's formula (Abbott 1925). The results were subject to log-probit-analysis (Finney 1971, Raymond 1985) and computered by Duncan's multiple range test and Student's *t*-test (Köhler et al. 1984). Data from the density test were treated by regression analysis.

Temperature effect: Bioassays with field-collected second and fourth instars of *Aedes vexans* (Meigen) were run at the following temperatures: 5, 8, 15 and 25 ± 0.5°C. Prior to their transfer to the rearing chamber, the larvae were acclimatized to the required temperature for 4 h and 25 of them were placed into each of the 200 ml plastic cups filled with 150 ml of distilled water at the required temperature. The range of *B.t.i.* concentrations used was 0.005–1.5 mg/

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liter. The mortality rates were determined after 48 hours.

Effect of larval density: Density tests were carried out with fourth instar larvae of *Aedes vexans* and *Culex pipiens* Linn. collected from the field. Densities of 10, 75, 150 and 200 larvae per 150 ml of distilled water were tested. This corresponds to densities of 67–1,330 specimens per liter. Doses ranged from 0.001–2.0 mg/liter.

Sunlight effects: The tests were conducted with late third instars of *Culex pipiens* and *Aedes aegypti*. *Culex* larvae were collected in the field whereas *Aedes aegypti* (Linn.) larvae (Bora Bora strain) were obtained from a laboratory culture. The tests were carried out in duplicate series: one exposed to sunlight for 7 h, and one simultaneously kept in the shade for the same time period.

The plastic cups containing 150 ml of distilled water and 25 larvae were placed outdoors into water baths and kept at a constant temperature of $25 \pm 1^\circ\text{C}$. The sunlight intensity was measured every hour by a Siemens exposure meter (M 09010-A 108), and ranged between 6,000 and 12,000 Lux depending on the time of day. The light intensity in the shade ranged from 80 to 300 Lux. After exposure for 7 h, both series were transferred to a rearing chamber without sunlight and kept for an additional 41 h at 25°C .

Impact of Daphnia: Two different densities of *D. curvirostris* (90 and 360 *Daphnia*/150 ml) were tested and compared with bioassays containing only *Aedes aegypti* larvae. The tests were run with a flowable concentrate (Teknar HP-D) at a range of 0.07–3.5 mg/liter and a powder formulation (Teknar TC) from 0.007–0.24 mg/liter.

RESULTS

Temperature: A comparison of the LC_{90} values showed that second instar larvae of *Aedes vexans* are about 10 times more sensitive to *B.t.i.* at 25°C ($\text{LC}_{90} = 0.014$ mg/liter) than at 5°C ($\text{LC}_{90} = 0.145$ mg/liter). At 8°C ($\text{LC}_{90} = 0.058$ mg/liter) and at 15°C ($\text{LC}_{90} = 0.062$ mg/liter) the activity of *B.t.i.* was 4 times less than at 25°C . There are no significant differences in activity between 8 and 15°C (Fig. 1).

Also no significant differences could be found between the LC_{50} values at 5°C ($\text{LC}_{50} = 0.022$ mg/liter) and at 15°C ($\text{LC}_{50} = 0.027$ mg/liter). But at 25°C ($\text{LC}_{50} = 0.002$ mg/liter) the activity of *B.t.i.* is about 13 times greater than at 15°C .

Like the second instar larvae, fourth instar larvae are about 9 times more sensitive at 25°C ($\text{LC}_{50} = 0.034$ mg/liter; $\text{LC}_{90} = 0.150$ mg/liter) than at 5°C ($\text{LC}_{50} = 0.318$ mg/liter; $\text{LC}_{90} = 1.338$ mg/liter). A significant increase in the activity

of *B.t.i.* from 6 times (LC_{50}) to 13 times (LC_{90}) could be observed between 5 and 8°C . On the other hand, the differences between 8 and 25°C were not significant (Fig. 2).

Density: Regression analyses on LC_{50} and LC_{90} values and the larval densities showed a significantly linear correlation for the 2 species tested (Figs. 3 and 4). In the case of *Ae. vexans* the LC_{90} values relative to 10 larvae/cup ($\text{LC}_{90} = 0.060$ mg/liter) were about 8 fold higher for 75 larvae/cup ($\text{LC}_{90} = 0.489$ mg/liter), about 18 fold higher for 150 larvae/cup ($\text{LC}_{90} = 1.077$ mg/liter) and about 26 fold higher for 200 larvae/cup ($\text{LC}_{90} = 1.534$ mg/liter). A comparison of the LC_{50} values gave similar results. At larval densities of 75, 150 or 200 per 150 ml, it was necessary to apply a dosage that was 7 times (0.111 mg/liter), 22 times (0.351 mg/liter) or 21

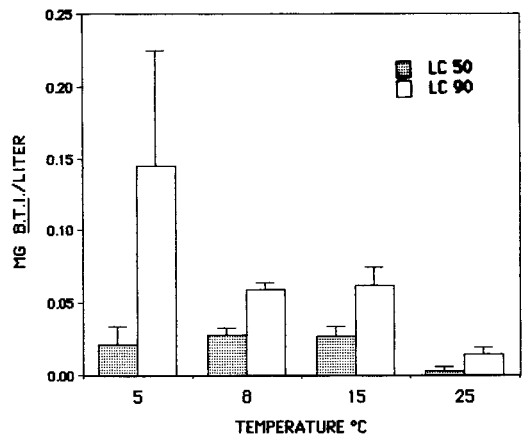


Fig. 1. Impact of temperature on *B.t.i.* efficacy against 2nd instar *Aedes vexans* larvae. Error bars indicate the SD.

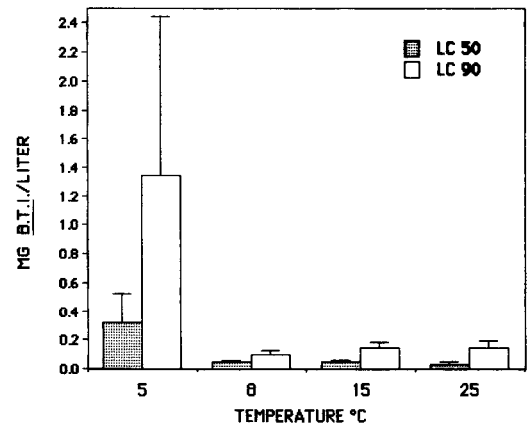


Fig. 2. Impact of temperature on *B.t.i.* efficacy against 4th instar *Aedes vexans* larvae. Error bars indicate the SD.

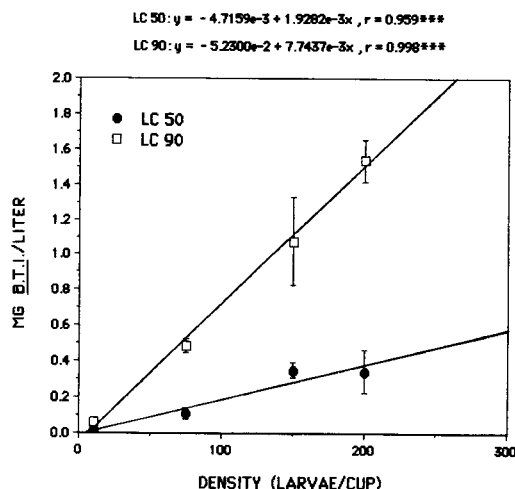


Fig. 3. Plot of *B.t.i.* concentration against *Aedes vexans* larval density. Error bars indicate the SD. Correlation coefficients for linear regression are highly significant ($P < 0.001$).

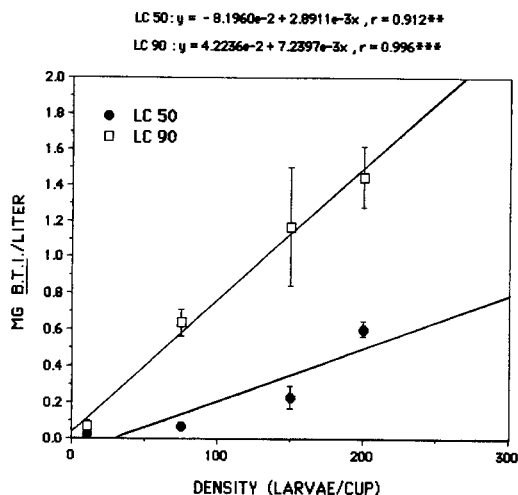


Fig. 4. Plot of *B.t.i.* concentration against *Culex pipiens* larval density. Error bars indicate the SD. Correlation coefficients for linear regression are significant ($P < 0.01$) or highly significant ($P < 0.001$).

times (0.342 mg/liter) greater than was required for 10 larvae per 150 ml ($LC_{50} = 0.016$ mg/liter).

Tests with *Culex pipiens* gave similar results (Fig. 4). The LC_{90} values relative to 10 larvae/cup ($LC_{90} = 0.07$ mg/liter) were about 9 fold higher for 75 larvae/cup ($LC_{90} = 0.637$ mg/liter), about 17 fold higher for 150 larvae/cup ($LC_{90} = 1.17$ mg/liter) and about 21 fold higher for 200 larvae/cup (1.442 mg/liter).

Sunlight: Strong sunlight can have a negative effect on the activity of *B.t.i.* (Table 1). In the series of tests using *Culex pipiens*, LC_{50} and LC_{90}

values were 2.5 times (LC_{50}) or 4 times (LC_{90}) greater in the series exposed to sunlight than in those placed in the shade (shade: $LC_{50} = 0.007$ mg/liter, $LC_{90} = 0.054$ mg/liter; sunlight: $LC_{50} = 0.017$ mg/liter, $LC_{90} = 0.236$ mg/liter).

Similar results were obtained in the series of tests with *Aedes aegypti*. LC_{50} and LC_{90} values were 3 times greater in the series exposed to sunlight than in those placed in the shade (shade: $LC_{50} = 0.039$ mg/liter, $LC_{90} = 0.086$ mg/liter; sunlight: $LC_{50} = 0.125$ mg/liter, $LC_{90} = 0.255$ mg/liter).

The impact of *Daphnia*: With the 2 products being tested, both LC_{50} and LC_{90} values increased as the density of *D. curvirostris* increased (Table 2). In tests with Teknar HP-D, the LC_{50} and LC_{90} values were 5 and 6 times higher at a density of 90 *Daphnia*/150 ml than were those without *Daphnia*. When the density of *Daphnia* was 360/150 ml, the values were 11 and 13 times higher, in other words more than twice as higher as with 90/150 ml. These results were confirmed with the tests undertaken with Teknar TC.

DISCUSSION

This study confirms previous field observations on differences in the efficacy of *B.t.i.* treatments in various environmental conditions (Becker and Ludwig 1983, Mulla et al. 1990).

The tests to evaluate the effect of temperature showed that there are distinct differences in the efficacy of *B.t.i.* especially between 5 and 8°C in both second and fourth instar larvae of *Aedes vexans*. This effect may be due to the reduced filtration rate by larvae at very low water temperatures. While no significant differences could be found in fourth instar larvae at 8, 15 and 25°C, a significant increase in activity was observed in second instar larvae between 15 and 25°C. In terms of practical application, this means that greater quantities of *B.t.i.* are needed to be applied at temperatures below 8°C.

An increase in the number of larvae has a negative influence on the efficacy of a *B.t.i.* application (Mulla et al. 1990). Our investigation has shown that there is a linear correlation between larval density and the efficacy of *B.t.i.* in both *Aedes vexans* and *Culex pipiens*. In practice, this implies that the average larval density has to be determined before the optimal dosage for a routine treatment can be established. As water levels fall in flooded areas the area to be treated will be reduced, but larvae will be more concentrated in the remaining water bodies and a higher dosage of *B.t.i.* will be required.

Increased sunlight lowers the efficacy of *B.t.i.*, as has also been shown by Burke et al. (1983)

Table 1. Effect of sunlight on *B.t.i.* efficacy against *Culex pipiens* and *Aedes aegypti* larvae.

Species	LC	Sun	Shadow
<i>Cx. pipiens</i>	LC ₅₀	0.0175 ± 0.0016	0.0066 ± 0.0001
	LC ₉₀	0.2355 ± 0.0362	0.0538 ± 0.0085
<i>Ae. aegypti</i>	LC ₅₀	0.1252 ± 0.0082	0.0395 ± 0.0125
	LC ₉₀	0.2554 ± 0.0045	0.0884 ± 0.0185

Table 2. LC₅₀ and LC₉₀ (mean values ± SD) obtained in bioassays with *B.t.i.* formulation and *Aedes aegypti* in different densities of *Daphnia curvirostris*.

	LC ₅₀	LC ₉₀
Teknar HP-D		
Without <i>Daphnia</i>	0.135 ± 0.003 a	0.298 ± 0.080 a
90 <i>Daphnia</i> /150 ml	0.686 ± 0.144 b	1.754 ± 0.678 b
360 <i>Daphnia</i> /150 ml	1.450 ± 0.248 c	3.798 ± 0.852 c
Teknar TC		
Without <i>Daphnia</i>	0.015 ± 0.002 a	0.026 ± 0.006 a
90 <i>Daphnia</i> /150 ml	0.035 ± 0.002 b	0.080 ± 0.008 b
360 <i>Daphnia</i> /150 ml	0.104 ± 0.011 c	0.230 ± 0.009 c

Values followed by the same letter are not significantly different (*P* = 0.05).

and Morris (1983). Ionizing radiation, as from a ⁶⁰Co radiation source, for example, has been successfully used in Germany to sterilize *B.t.i.* preparations (Schnetter et al. 1983, Krieg 1986), without diminishing the efficacy of the preparations treated. However, natural sunlight can decrease the efficacy of *B.t.i.* This is of particular relevance and importance for field applications in the tropics.

The larvae of many mosquito species such as those of *Culex* and *Culiseta* are often associated with other filter-feeding organisms (Rettich 1983). Investigations with *Daphnia curvirostris* have shown that competition among filter feeders leads to a decrease in the concentration of d-endotoxin in the water. This is similar to the effect that has been observed during increases in the density of filter-feeding mosquito larvae.

As with traditional insecticides, the efficacy of *B.t.i.* is affected by environmental factors. But when these factors are taken into account, *B.t.i.* can be incorporated simply, successfully, and with full environmental safety into routine programs for the control of mosquito larvae, as numerous projects worldwide have already shown.

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